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A retrospective case–control study of the use of hormone-related supplements and association with breast cancer

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Hormone-related supplements (HRS), many of which contain phytoestrogens, are widely used to manage menopausal symptoms, yet their relationship with breast cancer risk has generally not been evaluated. We evaluated whether use of HRS was associated with breast cancer risk, using a population-based case–control study in 3 counties of the Philadelphia metropolitan area consisting of 949 breast cancer cases and 1,524 controls. Use of HRS varied significantly by race, with African American women being more likely than European American women to use any herbal preparation (19.2% *vs.* 14.7%, $p = 0.003$) as well as specific preparations including black cohosh (5.4% *vs.* 2.0%, $p = 0.003$), ginseng (12.5% *vs.* 7.9%, $p < 0.001$) and red clover (4.7% *vs.* 0.6%, $p < 0.001$). Use of black cohosh had a significant breast cancer protective effect (adjusted odds ratio 0.39, 95% CI: 0.22–0.70). This association was similar among women who reported use of either black cohosh or Remifemin (an herbal preparation derived from black cohosh; adjusted odds ratio 0.47, 95% CI: 0.27–0.82). The literature reports that black cohosh may be effective in treating menopausal symptoms, and has antiestrogenic, antiproliferative and antioxidant properties. Additional confirmatory studies are required to determine whether black cohosh could be used to prevent breast cancer.

Key words: breast cancer; prevention; complementary alternative medicine

Hormone-related supplements (HRS) are widely used by women for the management of menopausal symptoms. While the specific contents of these preparations are unregulated by the Federal government, they generally contain phytoestrogens and other compounds that are thought to mimic the effect of endogenous estrogens. Commonly used preparations include herbal extracts of black cohosh (*Cimicifuga racemosa*), dong quai (*Angelica sinensis*), ginseng (*Panax quinquefolius*), red clover (*Trifolium pretense*) and yam (*Discorea alata*). Also available are preparations labeled as Biestrogen (Biest), dehydroepiandrosterone (DHEA), daidzein, Estrovin, genistein, Isoflavone, Promensil, Rejuvex, Remifemin, soy medications, steroid creams and Triestrogen. Many of these preparations contain a variety of herb-derived compounds, including those listed earlier. However, due to lack of standardization and government oversight, the concentrations and composition of these compounds varies widely.

It has been suggested that women who have diets rich in phytoestrogens, including women from Asian countries, may be at decreased breast cancer risk.[1,2] However, epidemiological evidence that phytoestrogen consumption is associated with modified cancer risk is largely limited to food intake, and has not revealed associations of specific compounds with breast cancer risk or protection.[2,3] The mechanism of this putative breast cancer protective effect also remains unclear. It has been proposed that the relatively weaker estrogenic effect of phytoestrogens may compete with that of estradiol and have antiproliferative properties that decrease breast cancer risk.[1,4–6] However, there is also evidence that the estrogenic activity of some of these compounds, including binding to estrogen receptors (ERs),[7,8] may stimulate breast tumor cell proliferation at physiological concentrations,[9–12] and increase endogenous steroid hormone levels.[13] In addition, estrogenic effects are not consistent across all phytoestrogen-containing compounds. For example, black cohosh is consistently viewed as having antiestrogenic, antiproliferative and antioxidant properties (*e.g.*, Refs. 14 and 15). Thus, the mechanism of action of these compounds in relieving menopausal symptoms or their potential relationship with hormone-induced cancer risk remains unclear. To address these issues, we used a population-based case–control study of women in the Philadelphia region to evaluate whether commonly used HRS for management of menopausal symptoms were associated with breast cancer risk or protection.

Material and methods

Study design and data collection

We conducted a population-based case–control study with incident breast cancer cases and controls selected from the community using random-digit dialing (RDD), frequency matched to the cases on age and race. The source population for this study was residents of Philadelphia and Delaware Counties in Pennsylvania and of Camden County in New Jersey. Potentially eligible cases were African American or European American women, residing in these counties at the time of diagnosis, who were 50–79 years old and were newly diagnosed with breast cancer between July 1, 1999 and June 30, 2002. The cases were identified through active surveillance at 38 hospitals. Quarterly reviews of the Pennsylvania Cancer Registry lists were used to validate the completeness of our case ascertainment in Pennsylvania. Additional details of our study design can be found in Strom *et al.*[16]

Women were eligible to be a breast cancer case if a pathology report was compatible with a first primary, invasive, breast cancer of any stage (I, II, III), any grade and any tissue type (ductal, lobular, mucinous, papillary, mixed). Women with ductal carcinoma *in situ* (DCIS), lobular carcinoma *in situ* (LCIS) and other nonmalignant tumor types were excluded. Pathology reports and other parts of the medical records were abstracted and reviewed in order to validate the diagnosis. Information about tumor type, size, grade, degree of metastasis, lymph node involvement and hormone receptor reactivity was abstracted from the pathology reports onto standardized abstraction forms.

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Controls were selected by RDD from the same geographic regions as the cases, and frequency-matched to the cases on age (in 5-year age groups), race (European or African American; Hispanic women who reported their race as European or African American were eligible) and calendar date of interview (±3 months). Controls were selected by a survey research firm that used a strict single stage method in which every residential telephone number had an equal and known probability of selection. Control ascertainment occurred concurrently with case ascertainment over the period from July 1, 1999 to June 30, 2002. The original plan called for equal numbers of cases and controls. About halfway through the study, we decided to increase the control-to-case ratio to increase power and to relax the age-matching criteria to increase the number of African American controls.

To be eligible for inclusion, controls could not have a history of breast cancer. Additional eligibility criteria for both cases and controls included living in a noninstitutional setting, having a household telephone, ability to speak English and lacking severe cognitive, language or speech impairment. To minimize the potential bias related to selecting controls from among individuals who are frequently at home and who may be different from individuals who are frequently out of the house, we required up to 9 attempts at contact at multiple times of the day and days of the week. The interval between diagnosis and case ascertainment could not exceed 18 months, and the interval between ascertainment and contacting cases for the screening interview could not exceed 12 months. Women in the control group were interviewed within 12 months from the date of the RDD screening interview.

We ascertained 1,890 incident breast cancer cases who met the age, county, diagnostic, diagnosis date and race criteria. Of these, 8 were living in a nursing home, 44 did not speak English, 25 were not mentally or physically able to participate, 416 did not have physician consent, 125 were without correct address and/or phone number and 58 died before we could contact them. Another 234 refused, and 31 could not be interviewed before the study ended. Of the 1,214 cases who were eligible and accessible, 949 were interviewed (50% of ascertained, 78% of those eligible and accessible).

The survey research firm provided the names, addresses and telephone numbers for 2,381 potential RDD controls. Of these, 181 were ineligible because of age, gender, county, race or history of breast cancer. Of those remaining, 22 could not participate because of physical or mental impairments, 11 did not speak English, 5 were deceased, 199 could not be recontacted because they moved or changed their phone number and 439 refused prior to viewing the research materials. The remaining 1,524 completed the interview (64% of those referred or 78% of those eligible and accessible).

Telephone interviews were used to collect data on demographic characteristics, family history of breast, endometrial and ovarian cancer, contraceptive history, fertility history, menstrual and menopausal history, medical history, detailed gynecologic screening history, use of exogenous hormones and use of other medications. The names of HRS commonly used for menopausal symptoms were included on a card mailed in advance to the study participants. We specifically asked about the use of Biest, black cohosh, DHEA, daidzein, dong quai, Estrovin, genistein, ginseng, Isoflavones, Promensil, red clover, Rejuvex, Remifemin, soy medications, steroid creams, Triestrogen and yam creams. During the interview, respondents could list up to 5 of these HRS used at least 3 times a week for 1 month or more any time before the reference date, which was defined as the date of diagnosis for the cases and the date of completion of the RDD screening for the controls.

Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the relationship of self-reported herbal preparation use and breast cancer. Multiple conditional logistic regression was performed to adjust simultaneously for the matching variables (defined by combinations of age group and race) and known risk factors for breast cancer. All models were adjusted by the same set of potential confounders: (*i*) education (less than high school, high school grad, greater than high school but not a college graduate or college graduate or higher), (*ii*) age at first full-term pregnancy (nulliparous *vs.* age of first live birth <20 *vs.* age at first live birth 20–24 *vs.* age of first live birth 25–29 *vs.* age of first live birth >30), (*iii*) menopause status (known natural, assumed natural at reference age of 50 if menopausal status is unknown and induced), (*iv*) family history of breast cancer (any *vs.* none), (*v*) reference age as a continuous variable and (*vi*) ever use of hormone replacement therapy. Other variables that were considered as confounders but were not significant predictors and did not change the point estimate associated with herb use by more than 10% in any analysis included age at menopause, use of oral contraceptives, body mass index, smoking history, history of bilateral oophorectomy and years of menses. Although we asked women to report their use of HRS only prior to diagnosis, we considered the potential for bias in use of HRS in the interval from time from diagnosis or ascertainment until interview. For this reason, we also included the interval from diagnosis (in cases) or ascertainment (in controls) to interview as a quartile range (<86 days *vs.* 87–135 days *vs.* 136–208 days *vs.* >209 days) as a potential confounder.

All analyses were performed in STATA (version 9.0, STATA Corporation, College Station, TX).

TABLE I – DEMOGRAPHIC CHARACTERISTICS OF BREAST CANCER CASES AND MATCHED RDD CONTROLS IN THE PHILADELPHIA AREA, 1999–2002

Characteristic	Cases (*N*)	Controls (*N*)	*p* value
Age at reference date, yr (mean ± SD)	63.0 (8.1)	61.8 (8.5)	<0.001
Race			<0.001
White	677 (43)[1]	905 (57)	
Black	272 (31)	619 (69)	
Marital status			0.085
Never married	93 (44)	119 (56)	
Ever married	854 (38)	1,402 (62)	
Highest schooling			0.453
Less than high school	138 (39)	220 (61)	
High school diploma	399 (40)	595 (60)	
Greater than high school diploma	201 (36)	352 (64)	
College degree	210 (37)	355 (63)	
Household income (pretax)			0.025
<$15,000	155 (39)	245 (61)	
$15,000–$45,000	342 (36)	609 (64)	
$45,000–$75,000	188 (37)	323 (63)	
>$75,000	129 (40)	190 (60)	

[1]Values within parentheses indicate percentages.

Results

Tables I and II summarize the characteristics of our study population. As expected, there was a difference in age and race by case–control status, and all subsequent analyses considered age and race to correct for potential confounding by these factors. Marital status and education were not different by case status, but income level was. Table II demonstrates that many known breast cancer risk factors including menopausal status, parity and family history were also shown to be breast cancer risk factors in this study. However, the sample size was small when stratified by HRS use to make strong inferences about the effect of some of these factors in HRS users.

As shown in Table III, between 10 and 20% of women in our study reported use of any HRS. This proportion varied by race. Overall, African American women were more likely to report use of any HRS than European American women (19.2% *vs.* 14.7%, $p = 0.003$). This relationship was observed both among breast cancer cases and controls, with 16.9% of African American cases reporting any herbal preparation use compared with 11.4% of European

TABLE II – CHARACTERISTICS OF BREAST CANCER CASES AND MATCHED RDD CONTROLS IN THE PHILADELPHIA AREA

Characteristic	No HRS use			HRS use		
	Cases (*N*)	Controls (*N*)	*p* value	Cases (*N*)	Controls (*N*)	*p* value
Body mass index (BMI)[1]			0.089			0.118
BMI < 30	740 (40)[2]	1,090 (60)		110 (32)	236 (68)	
BMI ≥ 30	80 (35)	151 (65)		12 (21)	44 (79)	
Age at first menarche			0.075			0.310
Early (≤12)	203 (43)	264 (57)		30 (25)	92 (75)	
Late (≥13)	619 (39)	973 (61)		56 (20)	223 (80)	
Imputed age at menopause[3]			0.371			0.603
Early (≤50)	417 (39)	654 (61)		66 (30)	154 (70)	
Late (≥50)	344 (41)	496 (59)		45 (33)	93 (67)	
Full term pregnancy[4]			<0.001			0.004
Never	128 (58)	94 (42)		259 (93)	21 (7)	
Ever	698 (38)	1,147 (62)		102 (83)	21 (17)	
Oral contraceptive use			0.011			0.538
Never used	481 (43)	641 (57)		45 (32)	96 (68)	
Used <3 yr	167 (38)	278 (62)		28 (26)	78 (74)	
Used >3 yr	176 (36)	319 (64)		50 (32)	104 (68)	
Hormone replacement therapy use			0.335			0.280
Never	473 (41)	684 (59)		53 (28)	137 (72)	
Ever	353 (39)	557 (61)		70 (33)	143 (67)	
Cigarette smoker			0.594			0.900
Never	330 (39)	511 (61)		49 (30)	113 (70)	
Ever	495 (40)	730 (60)		74 (31)	166 (69)	
Family history of breast cancer (first degree relatives)			0.008			0.089
None	663 (39)	1,052 (61)		95 (29)	236 (71)	
Any	163 (46)	189 (54)		28 (39)	44 (61)	
Estrogen receptor positive	521	NA	–	71	NA	–
Progesterone receptor positive	441	NA	–	56	NA	–

[1]Based on woman's usual weight when over the age decade 30–39.–[2]Values within parentheses indicate percentages.–[3]Age at menopause was imputed where unknown based on age at first use of menopausal hormone replacement therapy, if available.–[4]Defined as a pregnancy longer than 26 weeks.

American cases ($p = 0.022$) and 20.2% of African American controls compared with 17.2% of European American controls ($p = 0.130$), although this was not statistically significant among controls. Among the more commonly used preparations, African American women were significantly more likely than European American women to use black cohosh (5.4% *vs.* 2.0%, $p = 0.003$), ginseng (12.5% *vs.* 7.9%, $p < 0.001$) and red clover (4.7% *vs.* 0.6%, $p < 0.001$). The use of most preparations was uncommon, with Biest, daidzein, DHEA, Estrovin, genistein, Isoflavone, Promensil, Rejuvex, Remifemin, steroid creams and yam creams being used by no more than ~1% of women in our sample (Table III). Although asked in our questionnaire, no woman reported having used daidzein.

We also explored whether additional factors were predictive of use of any herbal preparation. As expected, women who had ever used hormone replacement therapy ($p = 0.005$) or were postmenopausal ($p = 0.023$) were significantly more likely to have ever used an herbal preparation. Women who had attended college or had a college degree were also more likely to have used HRS than women who had a high school education or less ($p < 0.001$). Women who had used oral contraceptives were more likely to use HRS than women who had never used oral contraceptives ($p = 0.011$). A family history of breast cancer in a first degree relative was not associated with ever use of HRS ($p = 0.270$). Women who were ever pregnant were not more likely to have used HRS ($p = 0.505$), and there was no significant association with age at first full term pregnancy ($p = 0.088$).

Comparing women who did and did not use a particular herbal preparation, the risk of breast cancer risk was significantly lower among women who reported use of any HRS compared with women who reported no use (adjusted OR 0.65, 95% CI: 0.49, 0.87; Table III). Of individual preparations, only black cohosh was significantly associated with a decreased risk of breast cancer (adjusted OR 0.39, 95% CI: 0.22, 0.70). Black cohosh is also marketed under the brand name Remifemin. Thus, we considered reported use of either black cohosh or Remifemin. Six women reported Remifemin but no black cohosh use, 88 women reported black cohosh but not Remifemin use, and 7 women reported using both preparations. There was a protective association between breast cancer and use of either or both compounds (adjusted OR = 0.47, 95% CI: 0.27, 0.82).

Also shown in Table I are comparisons of women who used a specific herbal preparation with those who had never used any herbal preparation. The resulting inferences were identical to those obtained when comparing women who did and did not take a specific herbal preparation (see previous paragraph).

Use of tamoxifen or raloxifene may reduce breast cancer risk as well as induce menopausal symptoms, and some women using tamoxifen or raloxifene may use HRS to address these symptoms. Therefore, we also considered the use of HRS and use of tamoxifen or raloxifene prior to cancer diagnosis (*i.e.*, as a chemopreventive agent, not in treatment for cancer). The power to identify such associations was low, since only 35 women used both tamoxifen/raloxifene and any herbal preparation and only 8 women used both tamoxifen/raloxifene and black cohosh. No significant interaction between tamoxifen/raloxifene use and use of any herbal preparation ($p = 0.469$) or black cohosh use ($p = 0.997$) was observed. In subset analyses of women who never used tamoxifen or raloxifene, the association of any herbal preparation (OR = 0.52, 95% CI: 0.34, 0.79), black cohosh (OR = 0.29, 95% CI: 0.12, 0.71) or black cohosh ± Remifemin (OR = 0.34, 95% CI: 0.15, 0.81) persisted or became stronger. Ever use of tamoxifen or raloxifene was not a significant confounder in the relationship between use of HRS or black cohosh with breast cancer. In addition, the interval between reference date and interview did not change the inferences of our study for any analysis.

To further elucidate the potential mode of action of black cohosh and/or Remifemin on breast cancer protection, we explored whether the effect of these preparations on breast cancer risk reduction was associated with the hormone receptor characteristics of the tumor, namely ER and progesterone receptor (PR) status. ER/PR status was available on 786 (83%) of breast cancer cases. In this subset of cases, the effect of black cohosh and/or Remifemin persisted

TABLE III – ASSOCIATION OF HORMONE-RELATED SUPPLEMENT (HRS) USE AND BREAST CANCER IN A POPULATION-BASED SAMPLE OF WOMEN IN THE PHILADELPHIA METROPOLITAN AREA

Exposure	Use in European Americans		Use in African Americans		Ever use of specific herb *vs.* never use of specific HRS, OR[1]	Ever use of specific herb *vs.* never use of any HRS, OR[1]
	Cases (N = 677)	Controls (N = 905)	Cases (N = 272)	Controls (N = 619)		
Any HRS	77 (11.4)[2]	155 (17.2)	46 (16.9)	125 (20.2)	0.65 [0.49–0.87][3]	0.65 [0.49–0.87]
Any phytoestrogen	20 (3.0)	44 (4.9)	20 (7.4)	46 (7.4)	0.76 [0.48–1.21]	0.69 [0.43–1.11]
Any Isoflavone or genistein	9 (1.3)	19 (2.1)	2 (0.7)	8 (1.3)	0.74 [0.32–1.67]	0.67 [0.29–1.53]
Isoflavone	9 (1.3)	17 (1.9)	0	5 (0.8)	ND[4]	ND[4]
Genistein	0	2 (0.2)	2 (0.7)	3 (0.5)	ND[4]	ND[4]
Red clover	2 (0.3)	8 (0.9)	13 (4.8)	29 (4.7)	0.78 [0.38–1.61]	0.70 [0.33–1.47]
Soy medications	11 (1.6)	21 (2.3)	6 (2.2)	14 (2.2)	0.81 [0.39–1.67]	0.69 [0.33–1.44]
Black cohosh or Remifemin	15 (2.2)	36 (4.0)	10 (3.7)	40 (6.5)	0.47 [0.27–0.82]	0.44 [0.25–0.77]
Black cohosh	13 (1.9)	34 (3.8)	9 (3.3)	39 (6.3)	0.39 [0.22–0.70]	0.37 [0.20–0.66]
Remifemin	3 (0.4)	6 (0.7)	2 (0.7)	2 (0.3)	ND[4]	ND[4]
Biestrogen	0	1 (0.1)	0	0	ND[4]	ND[4]
DHEA	8 (1.2)	16 (1.8)	2 (0.7)	4 (0.7)	ND[4]	ND[4]
Dong quai	12 (1.8)	21 (2.3)	9 (3.3)	20 (3.2)	0.83 [0.43–1.59]	0.75 [0.39–1.45]
Estrovin	3 (0.4)	4 (0.4)	3 (1.1)	7 (1.1)	ND[4]	ND[4]
Ginseng	41 (6.1)	84 (9.3)	31 (11.4)	80 (12.9)	0.74 [0.53–1.06]	0.75 [0.53–1.06]
Promensil	1 (0.2)	4 (0.4)	0	2 (0.3)	ND[4]	ND[4]
Rejuvex	7 (1.0)	10 (1.1)	2 (0.7)	8 (1.3)	ND[4]	ND[4]
Steroid creams	6 (0.9)	13 (1.4)	1 (0.4)	3 (0.5)	ND[4]	ND[4]
Yam creams	5 (0.7)	10 (1.1)	1 (0.4)	4 (0.7)	ND[4]	ND[4]

[1]The odds ratio (OR) represents the relationship of herbal exposure and breast cancer risk as estimated from conditional logistic regression matched on age and race, and adjusted for the following variables: (*i*) education (less than high school, high school grad, greater than high school but not a college graduate, or college graduate or higher), (*ii*) age at first full-term pregnancy (nulliparous *vs.* age of first live birth <20 *vs.* age at first live birth 20–24 *vs.* age of first live birth 25–29 *vs.* age of first live birth >30), (*iii*) menopause status (known natural, assumed natural at reference age of 50 if menopausal status is unknown, and induced), (*iv*) family history of breast cancer (any *vs.* none), (*v*) time from diagnosis/ascertainment to interview (<86 days *vs.* 87–135 days *vs.* 136–208 days *vs.* >209 days), (*vi*) reference age as a continuous variable and (*vii*) ever use of hormone replacement therapy.–[2]Values within parentheses indicate percentages.–[3]Values within square brackets indicate 95% CIs.–[4]Odds ratio associations not undertaken due to limited number of women who used this preparation.

regardless of ER status (OR = 0.35, 95% CI: 0.13, 0.96 for ER negative and OR = 0.50, 95% CI: 0.26, 0.97 for ER positive tumors). In contrast, the effect of black cohosh and/or Remifemin varied by PR status: the effect was significant in PR positive tumors (OR = 0.36, 95% CI: 0.17, 0.78) but not in PR negative tumors (OR = 0.62, 95% CI: 0.30, 1.29). While very preliminary, these results suggest that the effect of black cohosh and/or Remifemin may be greater in the ER positive and PR positive groups than in ER negative or PR negative tumors, although these differences are not large. In addition, these data suggest that PR activity may be related to the breast cancer protective effects of black cohosh/Remifemin because the effect of black cohosh and/or Remifemin was greater in PR positive tumors than in PR negative tumors.

Discussion

With the serious health concerns that have been raised about the use of estrogen and progestin-containing hormone replacement therapy in recent years,[17] some women have turned to complementary/alternative medicines to alleviate symptoms of menopause. The long-range effects of these compounds have not been studied. Therefore, we present the first report that black cohosh confers a degree of protection from breast cancer, which represents a potentially important piece of information for women who take, or who might consider taking, these compounds. These results also have implications for future research related to breast cancer chemoprevention.

Black cohosh has been widely used in American and Chinese traditional medicine.[18] The present results are consistent with our knowledge of the biological actions of black cohosh. Black cohosh has been reported to have beneficial effects on menopausal symptoms in some randomized clinical trials[19,20] but not all.[21] While many phytoestrogens have proestrogenic properties, and bind the ER with a similar affinity as 17β-estradiol itself,[22] black cohosh may have antiestrogenic effects that inhibit breast carcinogenesis. Black cohosh has been inferred to be antiestrogenic by studies that show a lack of estrogen-induced proliferation of breast cancer cells,[14,15,23–25] showed no competitive binding to ERs nor regulation of estrogen-inducible genes,[7] and protected against cellular DNA damage caused by reactive oxygen species by acting as an antioxidant.[26] These data are consistent with the hypothesis that black cohosh may be associated with protection from breast cancer risk.

While preliminary, we have also reported that the effect of black cohosh and/or Remifemin may differ with respect to the PR status of breast tumors. Given the recent data suggesting that breast cancer risk is increased among women with exposure to progestins in combined HRT,[17] this provides a potentially interesting link with mechanisms of hormonally induced breast carcinogenesis. However, it is not clear that use of black cohosh or Remifemin would necessarily be associated with the hormonal status of the tumors. Black cohosh has hormonal effects, but its action in terms of breast cancer risk may also be related to its antiproliferative properties,[4–8] which may or may not be independent of hormone receptor status or other hormonal hallmarks of the tumor. Furthermore, it is possible that most breast tumors become estrogen/progesterone sensitive and lose these receptors at a later stage in tumorigenesis. Thus, the hormone-mediated effects of black cohosh may remain even in ER/PR negative women, if these effects are acting on normal breast cells, early preneoplastic breast cells or breast tumor cells that have yet to lose their hormone receptors.

Studies of HRS are difficult to undertake because of limitations in data collection and the potential for study biases. We collected information about major categories of "regular" HRS use as shown in Table III. Usage of at least 3 times weekly for at least 1 month was asked about to minimize misclassification of exposure. Nonetheless, HRS are known by a variety of names, and because they are unregulated, may contain a variety of components and different dosages of the advertised components. This more detailed information about specific brands or preparations was not collected, which may lead to misclassification of exposure in this report. Some of these components may be unknown to the women who used them, and there is the possibility that exposure was misclassified because of variability in the naming and content of preparations that include black cohosh as an ingredient. Alternative therapies containing black cohosh are made from its roots and rhizomes. These HRS are not regulated by the FDA. Thus, the specific

content of these preparations is not uniform, and it may be difficult to identify specific content and dosage of black cohosh in many preparations. Black cohosh extract is sold under a variety of labels containing doses of the triterpene glycoside 26-deoxyactein. Black cohosh is also sold in tablet form, including the brand Remifemin (Enzymatic Therapy, Green Bay, WI), containing 20 mg of root per tablet. However, it is not always clear what specific compounds or doses have been consumed, and therefore misclassification or reporting bias could limit the inferences made in this report.

Similarly, our study has the potential for differential recall bias between cases and controls. While we specifically asked women about their consumption of HRS prior to the time of cancer diagnosis or the reference date in controls, differential reporting of herbal preparation use by cancer status could have biased our results. Participants who refused participation did so before knowing about the questions regarding herbal preparation use. Therefore, knowledge of our hypotheses was unlikely to have affected participation, and it is unlikely that refusal was differential with respect to the hypotheses studied here. However, if potential controls who used HRS were more likely to agree to participate, and this participation was different than in cases, then our results could be biased toward a protective effect of herbal preparation use. Finally, there has been a suggestion that the timing of phytoestrogen exposure (*e.g.*, early in life) may influence the effects of these compounds on breast cancer risk.[27] While self-reported exposure to these agents was prediagnosis, we did not have data regarding timing of exposures. We did consider the interval from diagnosis in cases or reference date in controls to interview, and the adjustment for this variable did not affect the inferences found in Table I. Future studies should consider the timing and duration of black cohosh use in order to better determine dose–response relationships.

Other exposures may influence the associations described here. For example, use of antibiotics or other agents that may alter the gastrointestinal flora that metabolize phytoestrogens may influence the observed associations between HRS and breast cancer. Confounding by other factors, particularly diet and physical activity may have influenced our results. We have explored adjustments for smoking and alcohol consumption, which were not significant confounders in our analysis. However, these adjustments are not optimal surrogate confounders for other relevant factors and additional studies should consider whether diet, physical activity or other exposures confound the relationship reported here. Women who take black cohosh and other HRS may have diets rich in other compounds that influence breast cancer risk, thereby confounding the relationship of herb use with breast cancer risk. Similarly, women who use these compounds may have lifestyles that involve physical activity or other exposures that differ from women who do not. While we did not have detailed dietary or physical activity information in this study, we did attempt to adjust for possible confounders including smoking and alcohol consumption history. Neither of these factors was a significant confounder in our analysis. Indeed, the raw (unadjusted) OR association for black cohosh use was not substantially different than the adjusted OR in our final adjusted analysis.

In summary, black cohosh has been previously shown to have antiproliferative, antiestrogenic and antioxidant properties, and is effective in the management of menopausal symptoms. However, side effects of black cohosh use have been reported,[28] there have been suggestions that black cohosh may influence breast cancer severity phenotype in a mouse model,[29] and the specific content and dose of preparations containing black cohosh are not always known. Therefore, substantial additional research must be undertaken before it can be established that black cohosh, or some compound found in black cohosh, is a breast cancer chemopreventive agent. Furthermore, women may wish to seek guidance from their physician before using these compounds, and the data presented here do not suggest that use of black cohosh is an appropriate substitute for standard hormone replacement therapy.

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